

Application No. 09/857,448  
Amdt. Dated January 20, 2004  
Reply to Office Action of July 15, 2003  
Docket No. 0508-1073

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1-19. (cancelled)

20. (new) An oligomeric conjugate positively charged, comprising:

an oligomer with a polymerization degree (PD) from 5 to 36, formed from monomeric components having substituted and unsubstituted  $\text{NH}_3^+$ , wherein

a) said monomeric component is substituted in a ratio of at least 50%, said ratio being determined by nuclear magnetic resonance, by protonable residues, said residues being protonated in a weak acid medium, said protonation leading to a destabilization of a cellular membrane,

b) said protonable residues possess the following properties:

said protonable residues contain a functional group enabling them to be linked to said oligomer,

said protonable residues are not recognized as a recognition signal recognized by a cellular membrane receptor,

said protonable residues comprise at least one unsubstituted  $\text{NH}_3^+$  group,

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c) the  $\text{NH}_3^+$  of said monomers are optionally substituted by uncharged residues leading to a reduction of the number of positive charges in comparison to the same oligomer before substitution,

d) molecules constituting a recognition signal recognized by a membrane cellular receptor are optionally present:

by substitution of some of the  $\text{NH}_3^+$  of said monomers, on some of the uncharged residues leading to a reduction of the number of charges,

on some of said protonable residues leading to a destabilization of a cellular membrane, or

by substitution of the  $\text{NH}_3^+$  (if it is present) of said protonable residues leading to a destabilization of a cellular membrane, provided that the total number of the non-substituted  $\text{NH}_3^+$  functions is of at least 50% of the polymerization degree.

21. (new) The oligomeric conjugate according to claim 20, wherein the protonable residues leading to a destabilization of cellular membranes have a pK in aqueous medium lower than 8.0.

22. (new) The oligomeric conjugate complex according to claim 20, wherein said protonable residues are compounds selected from the group consisting of:

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imidazoles,  
quinolines,  
pterines, and  
pyridines.

23. (new) The oligomeric conjugate according to claim 20, wherein said protonable residues comprise alkylimidazoles in which the alkyl radical has from 1 to 10 carbon atoms, and only one nitrogen atom of the imidazole ring is substituted.

24. (new) The oligomeric conjugate according to claim 20, wherein the protonable residues leading to a destabilization of cellular membranes are selected from the group consisting of histidine, 4-carboxymethyl-imidazole, 3-(1-methyl-imidazol-4yl)-alanine, 3-(3-methyl-imidazol-4yl)-alanine, 2-carboxy-imidazole, histamine, 3-(imidazol-4yl)-L-lactic acid, 2-(1-methyl-imidazol-4yl)ethylamine, 2-(3-methyl-imidazol-4yl)ethylamine,  $\beta$ -alanyl-histidine-(carnosine), 7-chloro-4(amino-1-methylbutylamino)-quinoline, N4-(7-chloro-4-quinolinyl)-1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6-methoxy-quinoline (primaquine), N4-(6-methoxy-8-quinolinyl)-1,4-pentanediamine, quininic acid, quinoline carboxylic acid, pteric acid, nicotinic acid, and quinolinic acid.

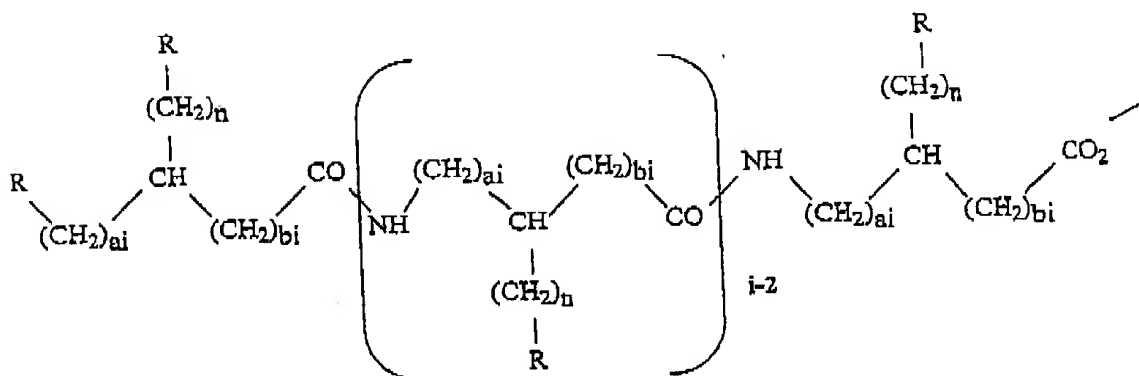
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25. (new) An oligomeric conjugate having the following formula:



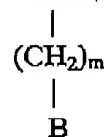
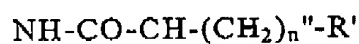
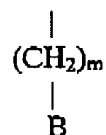
wherein: ai = an integer varying from 0 to 10,

bi = an integer varying from 0 to 10,

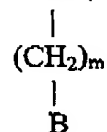
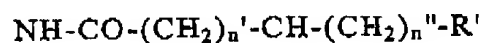
i = degree of polymerization from 5 to 36,

n = an integer varying from 1 to 6,

wherein 50% to 100% of all R groups are selected from the group consisting of



, and



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wherein  $m$  = an integer varying from 1 to 6,  
 $n'$  = an integer varying from 0 to 6,  
 $n''$  = an integer varying from 0 to 6,  
 $B$  = a weak base,  
 $R'$  represents  $\text{NH}_3^+$  (corresponding to a number  $p$ );  
or  $\text{NH}$  (corresponding to a number  $q$ ) substituted  
by a structure selected from the group consisting of

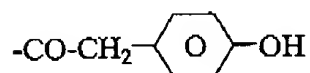
$-\text{CO}-\text{CH}_3$ ,

$-\text{CO}-(\text{CHOH})_r\text{H}$

$r$  being an integer from 1 to 15,

$-\text{CO}-(\text{CH}_2)_s-(\text{CHOH})_r\text{H}$

$r$  being an integer from 1 to 15, and  $s$  being an integer from 1 to 6,



$-\text{SO}_2\text{-Flu}$ ,

$-\text{CO-Flu}$ , and

$-\text{CS-NH-Flu}$

wherein  $\text{Flu}$  is a fluorescent molecule; and wherein  
0% to 50% of all  $R$  groups (corresponding to  $f$   
wherein:  $0 < f \leq u$ ) are

$\text{NH}_3^+$  (corresponding to a number  $j$ ); or

$\text{NH}$  (corresponding to a number  $k$ ), substituted by a  
structure selected from the group consisting of

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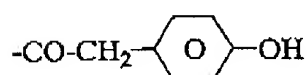
-CO-CH<sub>3</sub>,

-CO-(CHOH)<sub>r</sub>H

r being an integer from  
 1 to 15,

-CO-(CH<sub>2</sub>)<sub>s</sub>-(CHOH)<sub>r</sub>H

r being an integer from  
 1 to 15, and s being an  
 integer from 1 to 6,



-SO<sub>2</sub>-Flu,

-CO-Flu, and

-CS-NH-Flu wherein

Flu is a fluorescent molecule; or

H (corresponding to a number h); or

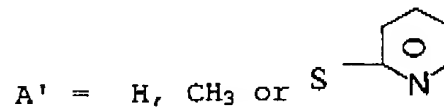
(CH<sub>2</sub>)<sub>n</sub>H

n being an integer from  
 1 to 6 (corresponding  
 to a number h); or

(CH<sub>2</sub>)<sub>n</sub>-OH

n being an integer from  
 1 to 6 (corresponding to  
 a number h); or

(CH<sub>2</sub>)<sub>n</sub>-SA'



n being integer from 1  
 to 6 (corresponding to a  
 number h)

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with  $i = u + j + k + h$

total number of  $\alpha \text{ NH}_3^+ = p = u - q$

total number of  $\omega \text{ NH}_3^+ = j = f - (k + h)$

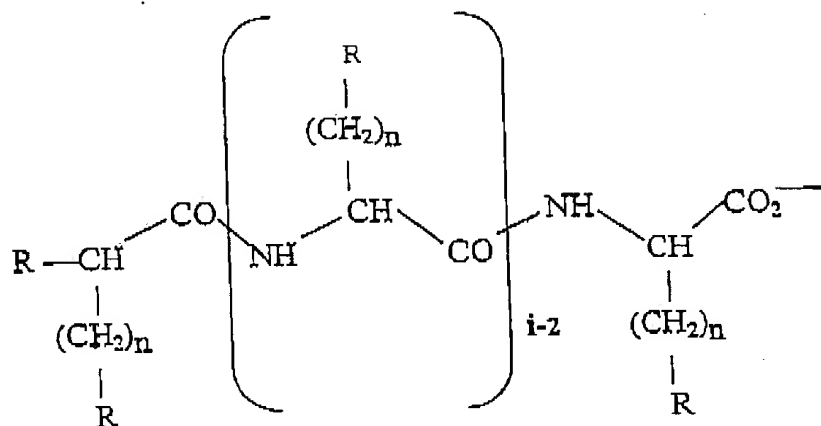
total number of  $\text{NH}_3^+ = m = p + j + 1$

with the proviso that:

1)  $u \geq i/2$

2)  $m \geq i/2$ .

26. (new) The oligomeric conjugate according to claim 25, wherein the oligomeric conjugate contains an oligomer of the following formula:



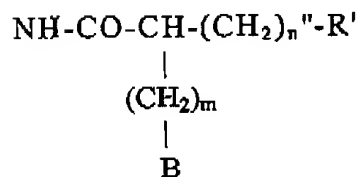
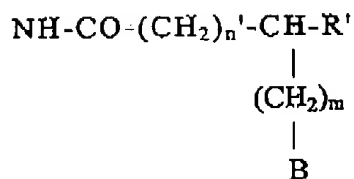
wherein

$i$  = degree of polymerization from 5 to 36,

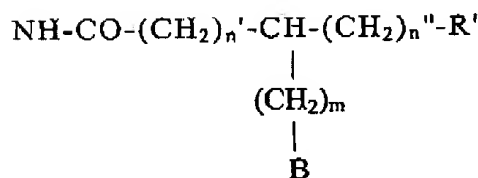
$n$  = is an integer varying from 1 to 6,

wherein 50% to 100% of all R groups (corresponding to  $u$ ) are selected from the group consisting of

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, and



m = an integer varying from 1 to 6,

n' = an integer varying from 0 to 6,

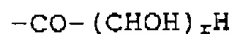
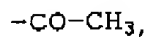
n'' = an integer varying from 0 to 6,

B = a weak base,

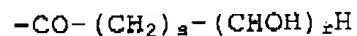
R' represents NH<sub>3</sub><sup>+</sup> (corresponding to a number p);

or NH (corresponding to a number q) substituted by a

structure selected from the group consisting of



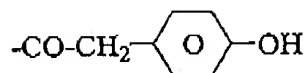
r being an integer from  
1 to 15,



r being an integer from  
1 to 15, and s being  
an integer from 1 to  
6,



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-SO<sub>2</sub>-Flu,

-CO-Flu, and

-CS-NH-Flu wherein

Flu is a fluorescent molecule;

and wherein 0% to 50% of all R groups (corresponding

to f: 0 < f ≤ u) are

NH<sub>3</sub><sup>+</sup> (corresponding to a number j); or

NH (corresponding to a number k), substituted by a

structure selected from the group consisting of

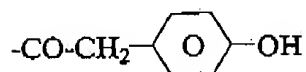
-CO-CH<sub>3</sub>,

-CO-(CHOH)<sub>r</sub>H

r being an integer from 1  
to 15,

-CO-(CH<sub>2</sub>)<sub>s</sub>-(CHOH)<sub>r</sub>H

r being an integer from 1  
to 15, and s being an  
integer from 1 to 6,



-SO<sub>2</sub>-Flu,

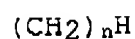
-CO-Flu, and

-CS-NH-Flu, wherein

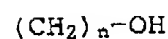
Flu is a fluorescent molecule; or

H (corresponding to a number h); or

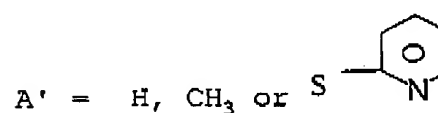
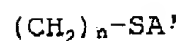
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n being an integer from  
 1 to 6 (corresponding  
 to a number h); or



n being an integer from  
 1 to 6 (corresponding to  
 a number h); or



n being integer from 1  
 to 6 (corresponding to a  
 number h)

with  $i = u + j + k + h$

total number of  $\alpha \text{ NH}_3^+ = p = u - q$

total number of  $\omega \text{ NH}_3^+ = j = f - (k + h)$

total number of  $\text{NH}_3^+ = m = p + j + 1$

with the proviso that:

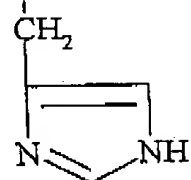
1)  $u \geq i/2$

2)  $m \geq i/2$ .

27. (new) The oligomeric conjugate according to claim  
 26, wherein said oligomeric conjugate is selected from the group  
 consisting of

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i = 19 n = 4 (u) R = NH-CO-CH-NH<sub>3</sub><sup>+</sup>



having the formula R = NH-CO-(CH<sub>2</sub>)<sub>n'</sub>-CH-(CH<sub>2</sub>)<sub>n''</sub>-R'  
 |  
 (CH<sub>2</sub>)<sub>m</sub>  
 |  
 B

wherein

n' = n'' = 0

R' = NH<sub>3</sub><sup>+</sup>

m = 1

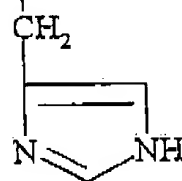
B = imidazole

(f) R = NH<sub>3</sub><sup>+</sup>

u = 12

j = 7,

i = 19 n = 4 (u) R = NH-CO-CH-NH<sub>3</sub><sup>+</sup>



having the formula R = NH-CO-(CH<sub>2</sub>)<sub>n'</sub>-CH-(CH<sub>2</sub>)<sub>n''</sub>-R'  
 |  
 (CH<sub>2</sub>)<sub>m</sub>  
 |  
 B

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wherein

$$n' = n'' = 0$$

$$R' = \text{NH}_3^+$$

$$m = 1$$

$$B = \text{imidazole}$$

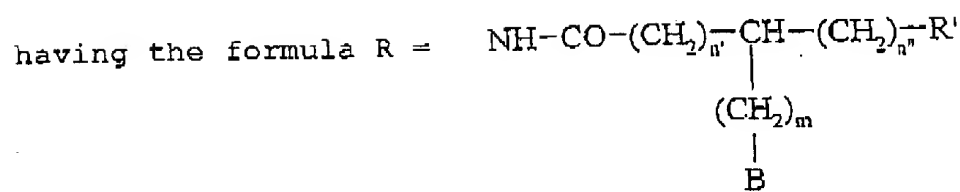
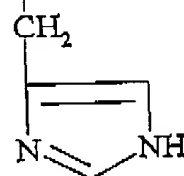
$$(f) R = \text{NH}_3^+$$

$$u = 16$$

$$j = 3,$$

or

$$i = 19 \quad n = 4 \quad (u) \quad R = \text{NH-CO-CH-NH}_3^+$$



wherein

$$n' = n'' = 0$$

$$R' = \text{NH}_3^+$$

$$m = 1$$

$$B = \text{imidazole}$$

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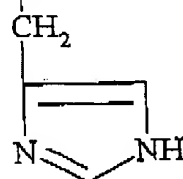
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(f)  $R = NH_3^+$

$u = 19$

$j = 0,$

$i = 19 \quad n = 4 \quad (u) \quad R = NH-CO-CH-NH_3^+$



having the formula  $R = NH-CO-(CH_2)_n-CH-(CH_2)_{n'}-R'$   
 $(CH_2)_m$   
 $B$

wherein

$n' = n'' = 0$

$R' = NH_3^+$

$m = 1$

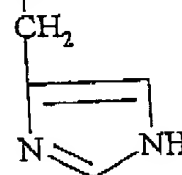
$B = \text{imidazole}$

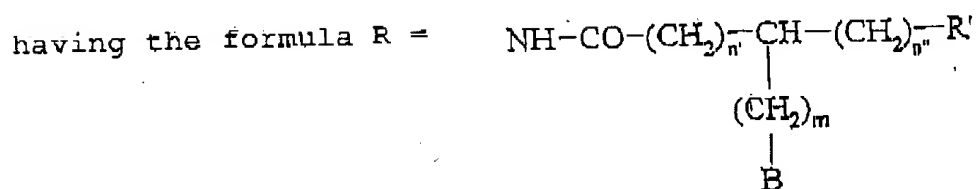
(f)  $R = CO-CH_3$

$u = 11$

$k = 8,$

$i = 19 \quad n = 4 \quad (u) \quad R = NH-CO-CH-NH_3^+$





wherein

$$n' = n'' = 0$$

$$\text{R}' = \text{NH}_3^+$$

$$m = 1$$

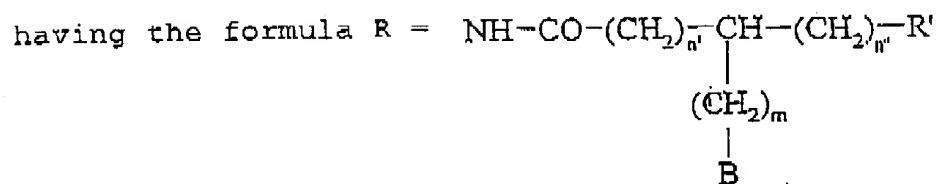
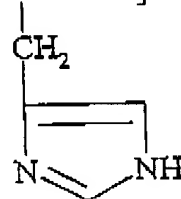
$$\text{B} = \text{imidazole}$$

$$(f) \text{ R} = \text{CO-CH}_3$$

$$u = 15$$

$$k = 4,$$

$$i = 19 \quad n = 4 \quad (u) \quad \text{R} = \text{NH-CO-CH-NH}_3^+$$



wherein

$$n' = n'' = 0$$

$$\text{R}' = \text{NH}_3^+$$

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$$m = 1$$

B = imidazole

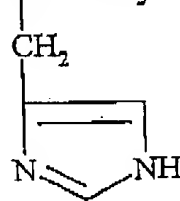
(f) R = CO-(CHOH)<sub>r</sub>H

$$r = 5$$

$$u = 12$$

k = 3, and

i = 19 n = 4 (u) R = NH-CO-CH-NH<sub>3</sub><sup>+</sup>



having the formula R = NH-CO-(CH<sub>2</sub>)<sub>n'</sub>-CH-(CH<sub>2</sub>)<sub>n''</sub>-R'  

$$\begin{array}{c} | \\ (CH_2)_m \\ | \\ B \end{array}$$

wherein

(q) n' = n'' = 0

R' = NH-CO-CH<sub>3</sub>

$$m = 1$$

B = imidazole

(f) R = NH<sub>3</sub><sup>+</sup>

$$u = 16$$

$$f = 4$$

$$k = 3.$$

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28. (new) A composition comprising an oligomeric conjugate according to claim 27, in association with an oligonucleotide.

29. (new) A combined preparation, in the form of a kit-of-parts, comprising:

- a) an oligomeric conjugate according to claim 20, and
- b) an oligonucleotide for the simultaneous, separate or sequential use, for the *in vitro*, *in vivo*, or *ex vivo* transfer of said biological molecules into a cytosol and/or cell nucleus.

30. (new) A method for the *in vitro*, *ex vivo*, or *in vivo* intracellular transfer of oligonucleotides into a cytosol and/or in a cell nucleus of a cell, comprising:

treating said cell with an oligomeric conjugate according to claim 20 in association with an oligonucleotide.

31. (new) A method for the *in vitro*, *ex vivo*, or *in vivo* transfer of an oligonucleotide, into a cytosol and/or a cell nucleus of a cell, comprising:

treating said cell with an oligomeric conjugate according to claim 20 in association with said oligonucleotide.

32. (new) The method according to claim 30, wherein the cells are selected from the group consisting of muscular, epithelial, endothelial, and myeloid cells.



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33. (new) A pharmaceutical composition, comprising as an active substance, an oligomeric conjugate according to claim 20, in association with a pharmaceutically acceptable vehicle.

34. (new) A kit or case comprising:

- a) an oligomeric conjugate according to claim 20,
- b) at least one biological molecule to transfer, and
- c) reagents enabling transfer of at least one biological molecule into a cell.

35. (new) An oligomeric conjugate positively charged, comprising:

an oligomer with a polymerization degree (PD) from 5 to 36, formed from monomeric components having free  $\text{NH}_3^+$  and substituted  $\text{NH}_3^+$ , wherein

a) the free  $\text{NH}_3^+$  of said monomeric components are in a ratio of at least 50%, said ratio being determined by nuclear magnetic resonance, by protonable residues, said residues being protonated in a weak acid medium, said protonation leading to a destabilization of a cellular membrane,

b) said protonable residues possess the following properties:

said protonable residues contain a functional group enabling them to be linked to said oligomer,

said protonable residues are not recognized as a recognition signal recognized by a cellular membrane receptor,

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said protonable residues comprise at least one free  $\text{NH}_3^+$  group,

c) the free  $\text{NH}_3^+$  of said monomers can optionally be substituted by uncharged residues leading to a reduction of the number of positive charges in comparison to the same oligomer before substitution,

d) molecules constituting a recognition signal recognized by a membrane cellular receptor may also optionally be present

by substitution of some of the free  $\text{NH}_3^+$  of said monomers,

on some of the uncharged residues leading to a reduction of the number of charges,

on some of said protonable residues leading to a destabilization of a cellular membrane, or

by substitution of a free  $\text{NH}_3^+$  of said protonable residues leading to a destabilization of a cellular membrane.